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ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 50, nr 4: 625—636
1981

Nonparallelism of cambium cells in neighboring rows

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(Received: February 4, 1981)

Abstract

The periclinal walls of cambial cells in neighboring lineages (rows) may not be parallel when viewed in their radial aspect. This lack of longitudinal parallelism may be so extensive that in active cambium pairs of cells from neighboring rows may be in contact only along restricted segments. This means that the initial cells, rather than forming a continuous layer, may be arranged in an irregular network pattern from which some parts project inward or outward from the layer of their mutual contacts. The longitudinal nonparallelism of cambial cells becomes more pronounced during symplastic radial growth. Unequal periclinal divisions counteract this, and in initial cells abscission of the parts projecting from the layer of mutual contact occurs. When the cambium passes from a period of activity to a period of rest a continuous layer of initials is reestablished. This involves elongation by intrusive growth of those cells previously shortened as the result of irregular periclinal divisions. The division walls in cambial cells may be warped, that is they change their orientation along the longitudinal direction perhaps even similar to an aircraft propeller. A division wall may thus be periclinal in one part of the cell and anticlinal in another.

INTRODUCTION

The term "cambium" has two meanings. In the wider sense "cambium" refers to that lateral meristem which can be distinguished as a multicellular layer of dividing, nondifferentiated cells between the phloem and xylem. In this sense cambium is an anatomical notion; a layer of such cells can be distinguished by anatomical methods. In the narrower sense, "cambium" is a unicellular layer of initials (Wilson et al. 1966). In this sense "cambium cell" means the same as "initial cell", the latter being, however, an anatomical-developmental notion. An initial in a group of presently existing cells is, specifically, a cell which will or can give rise to such a complete group in the future.

An initial may, but need not, differ morphologically from other cells. If, as is generally true in cambium, the initial cell does not differ (Srivastava and O'Brien 1966, Murmanis 1970, 1971, Barnett 1973), then it can be distinguished only through investigations of cambial development.

The author believes that cambium should be considered as an anatomical-morphological notion not only for sparing terminology, but also in view of the results presented in this paper. It appears that a fusiform cell in one row of the undifferentiated cells between the xylem and phloem may come into contact with different cells of the neighboring rows at different levels. Thus, even if the initial cells of two neighboring rows are in lateral contact at one level, they may be out of contact at other levels. This study was focused on the mutual positions of fusiform cambial and on their division. Effects of nonparallel arrangements of initial cells in adjacent rows were sought. The question of how cells in neighboring rows can grow radially without gliding was confronted. All study materials were from *Picea abies*.

The aim of this research was not to challenge the commonly applied definition of cambium as a layer of fusiform initials but to learn what latitude of meaning must be given to the term "layer" if this definition is to be rigorous.

A particularly convenient material for investigations of cambial development at the cellular level is the cambium occurring in tumors appearing as small thickenings on stems and branches, and similar to those described by White and Millington (1954) in *Picea glauca*. The cellular developmental processes in such tumorous cambium are exaggerated in their intensity and rapidity, which facilitates their study (Włoch 1976).

MATERIAL AND METHODS

The study material consisted of normal and tumorous cambium from *Picea abies* stems. The cambium was collected from various-aged trees in June when it showed maximum activity and in winter when it was inactive.

Semithin sections (ca. 3 μm) of cambium were prepared as for examination with the electron microscope. They were fixed in 2-4 per cent glutaraldehyde buffered at pH 7.3 with 0.2 M phosphate buffer, dehydrated in an acetone gradient and propylene oxide, and saturated with Epon. The latter was prepared according to Luft (1961). The material thus prepared was placed in gelatin capsules and, after polymerization was cut on an ultramicrotome. The successive semithin sections were put on microscopic slides and left to dry for two days.

The sections were stained with leucofuchsin (PAS reaction) and toluidine blue. The initial material for analysis consisted of successive sections through the cambium. Series of photomicrographs were taken of the sections. The photographs and the sections were the materials investigated.

RESULTS

On radial sections, and also on series of transverse sections, of tumorous cambium from spruce trees it is seen that the periclinal walls of cambial cells of one radial row cross the periclinal walls of cells of the neighboring row at a sharp angle. Because of this the fusiform cells in neighboring rows are not parallel. A cell of one row comes into contact with various cells of the neighboring row at different levels.

Figure 1 is a diagram of the position of periclinal walls in two neighboring rows in the tumorous cambium zone in projection on a radial plane. Positional information was taken from a series of transverse sections. The position of the periclinal walls at the site of contact between cells in the two rows is shown in the diagram. The region examined comprised two rows of cells in a segment extending 380 μm in the longitudinal direction and 150 μm in the radial direction. Thus, only parts of the cells are included in the diagram. In the radial cell row denoted by the broken lines the ends of several cells can be seen, although most cell ends of this row were located at a distance of about 1 mm above and below the studied area. These ends do not occur continuously in the radial row (next to the longer cells a shorter one appears), thus they must have been formed as the result of unequal periclinal division. If in this cambium we follow the course of one cell in a given row, for instance cell no. 12 (hatched), we observe that at various levels (A-J) along the long axis this cell contacts successively cells nos. 9, 10, 11, 11′, 12 and then once again cells 11′, 11 and 10 of the neighboring row. This illustrates our observation that a single cambial cell can contact various fusiform cells of the neighboring rows. Precise drawings of cross sections through the studied rows are shown in Fig. 2, corresponding to the sites which in Fig. 1 are marked by broken lines denoted by successive letters.

Periclinal walls may be crossed in normal cambium also, but usually not to such an extent as in tumorous cambium.

The crossing of fusiform cells in adjacent rows is increasingly pronounced with increasing thickness and activity in the cambial zone. Such crossing is less in thinner cambia and least in resting cambia. In normal resting cambium a cell can usually be distinguished in each row which contacts along its whole length one cell of the neighboring

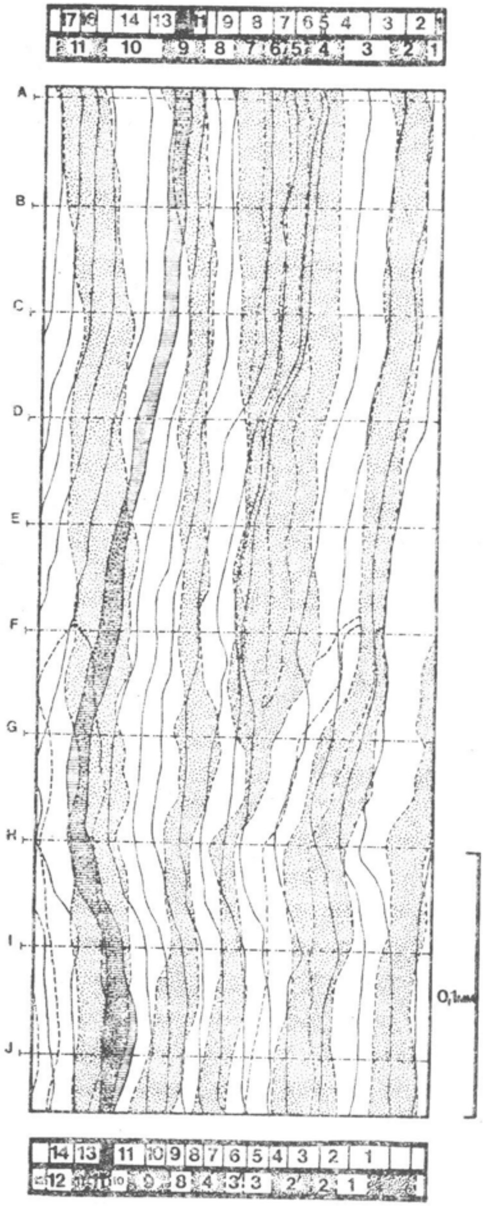

Fig. 1. Diagram plotted from a series of cross sections of tumorous *Picea abies* cambium, showing the arrangement of periclinal walls at the site of contact of two neighboring rows of cells in the cambial zone. The cells of the nearer row are denoted by a continuous line and those of the more distant row with a broken line. At the top and bottom of the diagram the radial rows examined are marked in cross section, numbered successively from the phloem side. The transverse dashed lines denoted by successive latters show the levels for which drawings are presented in Fig. 2. In the further row some groups of cells are marked by stippling to improve clarity of the diagram. Cell no. 12 in the nearer row is hatched in order to expose more clearly its contacts with various cells in the neighboring row

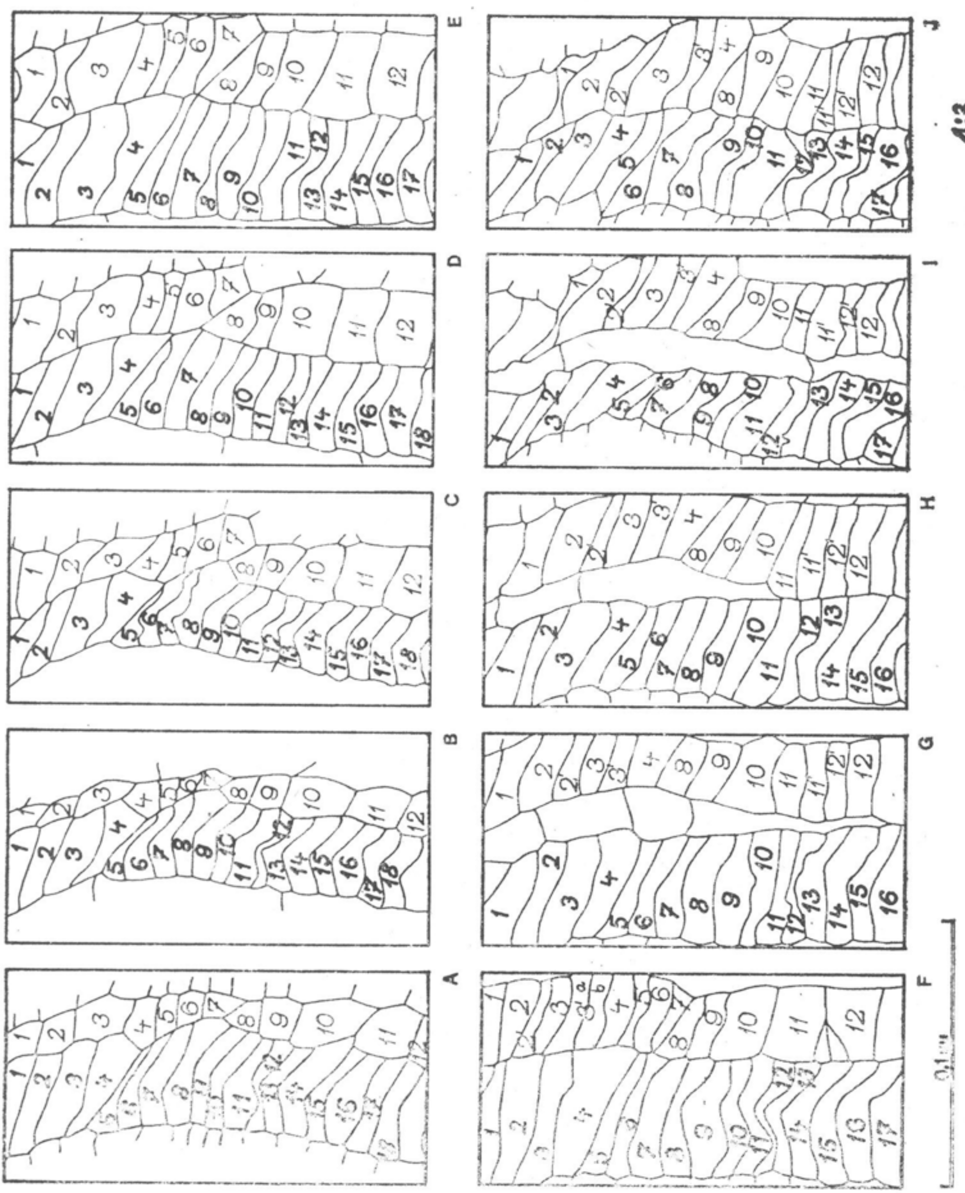

Fig. 2. Drawings of specified cross sections of the two radial rows of cells shown in Fig. 1. The various drawings in this figure correspond to the levels in Fig. 2 denoted by the same letters. Bold faced figures denote cells of the row in Fig. 1 marked by a continuous line, normal figures denote cells marked by a dashed line

radial row. These cells in tangentially contiguous rows form a continuous layer. This layer corresponds in its position to that of initial cells.

CELL DIVISION AND THE NONPARALLELISM OF CAMBIUM CELLS

Apart from periclinal divisions of mother cells into two cells of equal length, which is the same as that of the mother cell, there occur in tumorous cambium divisions with abnormal wall orientation, which cause a reconstruction of the whole cell arrangement. To these belong: a) unequal periclinal partitions and b) partitions with twisted orientation of partition walls.

Unequal periclinal partitions

Frequently in tumorous cambium, and sometimes in normal cambium, unequal periclinal partitions occur. These divide a mother cell into a longer and a shorter daughter cell. Cells resulting from unequal periclinal partitions are shown in Fig. 1 (cells nos. 2, 3, 4, 5, 6, 7, 11 and 12 in the layer of cells denoted by the broken line, specifically at levels F and G). Sometimes the partitioning wall is very short, as

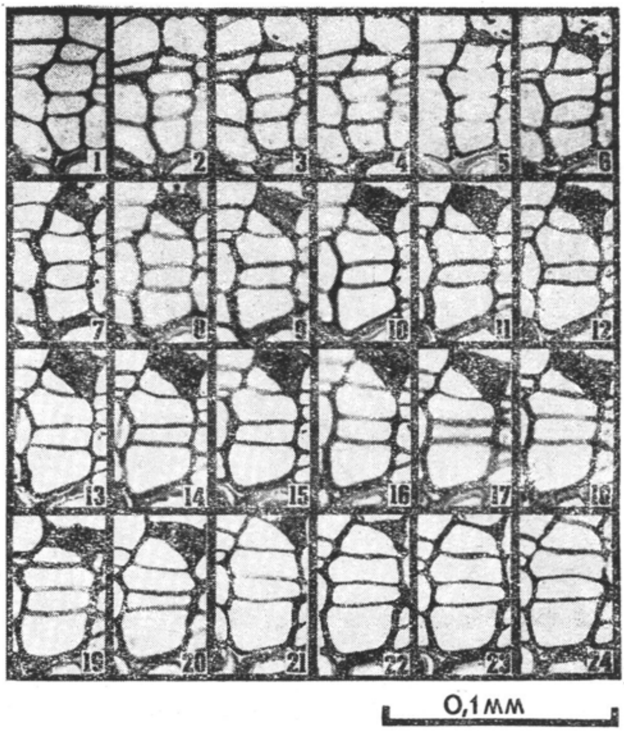

Fig. 3. Series of successive cross sections of 3 μm thickness through resting cambium. A short cell (hatched) appears in photomicrograph no. 3. Its other end is seen in photo no. 23. This is an example of a periclinal lateral wall partitioning off a short cell. This wall arose on the phloem side of the mother cell

in Fig. 1 in cell no. 3 in the layer denoted by a broken line (level F). Such a wall divides the mother cell into two shorter ones, an upper and a lower one, as does the wall in pseudotransverse anticlinal divisions. The difference between pseudotransverse anticlinal and periclinal oblique division is then only a difference of orientation of the plane of the partitioning wall in relation to the cambial plane. The partitioning wall frequently begins and ends on the same periclinal wall of the mother cell. Such lateral divisions produce one short daughter cell and one of the same length as the mother cell. The lateral cell arises either on the phloem side or the xylem side of the mother cell. On cross sections the wall that partitions off the lateral cell may be slanting to the mean cambial surface (Fig. 3).

Twisting of partition walls

A twisted partition wall is one having a shape superficially suggesting an aircraft propeller. A cell division in which a twisted partition wall is formed may be anticlinal at one end and periclinal at the other. Along some segments of its length the partition the wall may assume an oblique position so that along one side it comes into contact with the radial wall and along the other side with the periclinal wall. Twisted dividing walls are visible on a series of successive cross sections through the cambium in Figs. 4 and 5.

In the series of cross sections shown in Fig. 4 it can be observed that:

1. A partitioning wall can change its orientation along its longitudinal course and that an anticlinal wall (photomicrographs 1-17) becomes periclinal in its further course (photomicrographs 33-54).
2. Twisted partitions do occur in cells recognizable as initial cells.
3. Twisted partitions which are periclinal at one end and anticlinal at the other can produce two daughter cells of different length. This is due to the partly pseudotransverse character of such partitions.

In the above described cases the twisting of the wall did not exceed 90° (Fig. 5).

In most of the cases examined it seemed that the twisting of the partition wall originated in the cell division in which this wall was formed. It is clear, however, that slight changes in wall orientation may occur because of unequal rates of radial growth of corresponding opposite segments of radial walls after periclinal division. A change in wall orientation close to a cell end may occur during intrusive growth of that end.

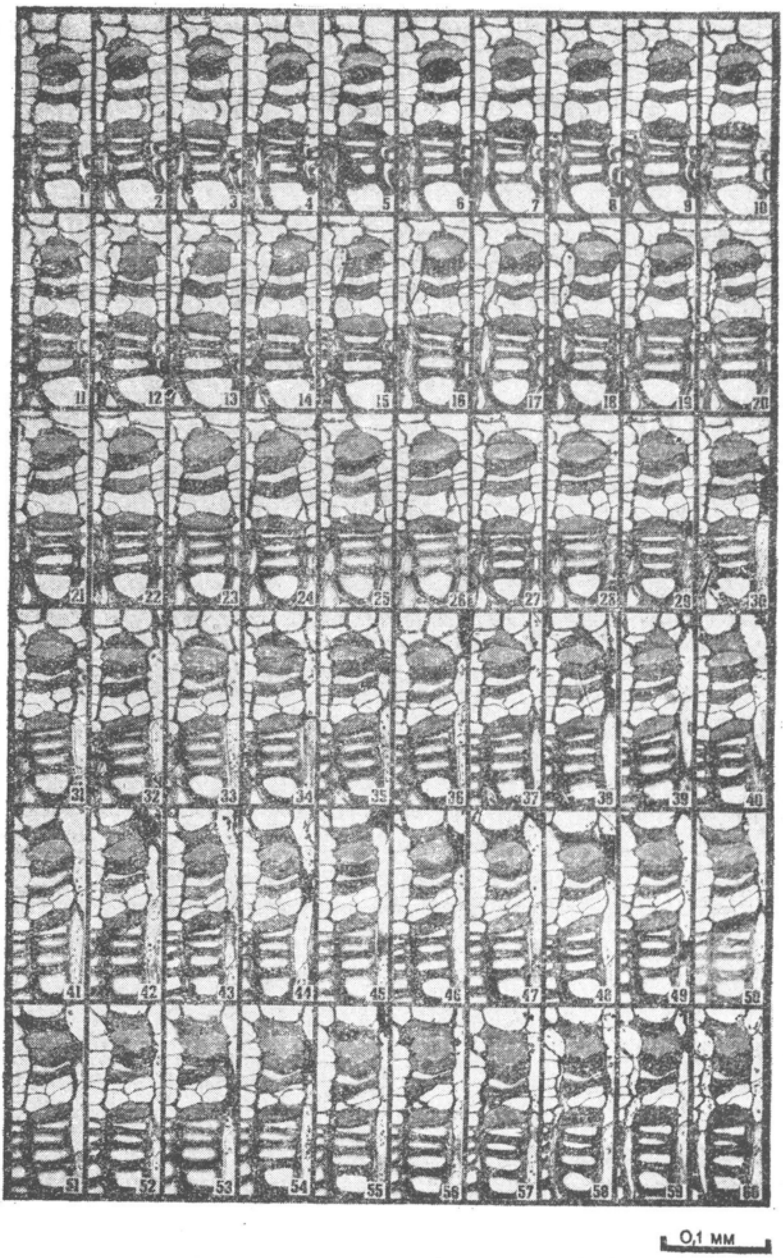

Fig. 4. Series of 60 cross sections through cambium in the resting condition. The successive photos show every second section (3 μm thick). The whole comprises a segment 360 μm long. Some cells are hatched to accentuate the second (clear) cambium cell counted from the xylem side. In this cell anticlinal division occured at the upper end (photos nos. 2-17), then the dividing wall was twisted to the left, changing its orientation to periclinal (photos nos. 33-54). A second twisted division in the lower part of the mother cell is visible in photos nos. 24-60

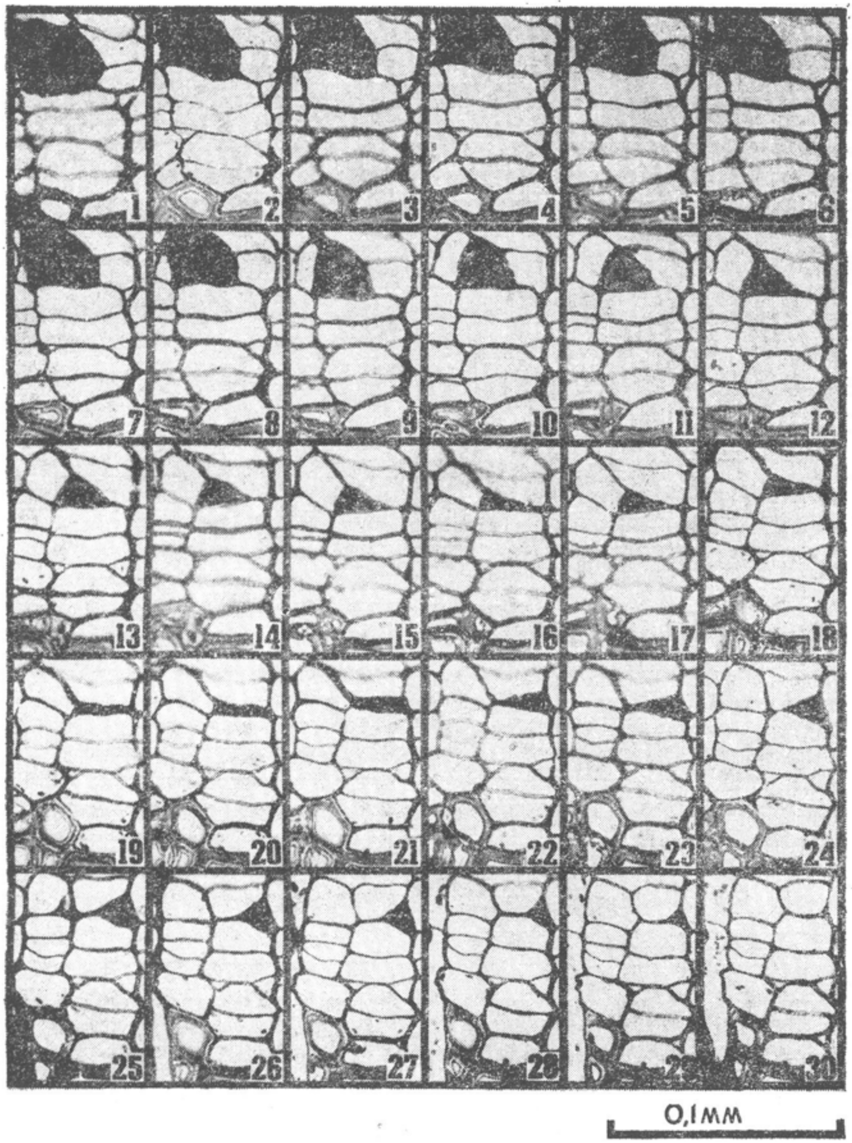

Fig. 5 .Series of successive cross sections 3 μm thick through resting cambium. The anticlinal wall on the right side of the hatched cell (photo no. 1) changes its orientation along its downward course to periclinal (photos nos. 10-20) and ends in the right, lower corner on the xylem side (photos nos. 21-30). This wall is twisted by more than 90°

DISCUSSION

The crossing at acute angles of periclinal walls of fusiform cells in neighboring radial rows is the consequence of nonparallel periclinal division in these rows. Cambium consists of radial cell rows held together by their middle lamellae and growing in a coordinated manner in the radial direction. This coordinated growth causes an increase in the angle between the crossed cells as radial growth advances (Fig. 6). If the subsequent periclinal partitions would run from one end of the cells to the other and divide the cells into equal parts, the crossing of cells in neighboring rows would become more frequent as the cells grow. This probably happens during the first phase of the seasonal activity of the cambium. The state of cell crossing attained in this way increases the number of contacts between the cells of the future xylem. However, if compensating factors did not come into play, one end of a cell could lie in the phloem and the other end in the xylem. The mechanism counteracting such extreme divergence of the fusiform cell ends, particularly of initial cells, is interesting. Crossing of cells due to coordinated radial growth is counteracted by the unequal periclinal divisions followed by intrusive growth. These phenomena are analogous to those that change cell arrangements in the tangential plane.

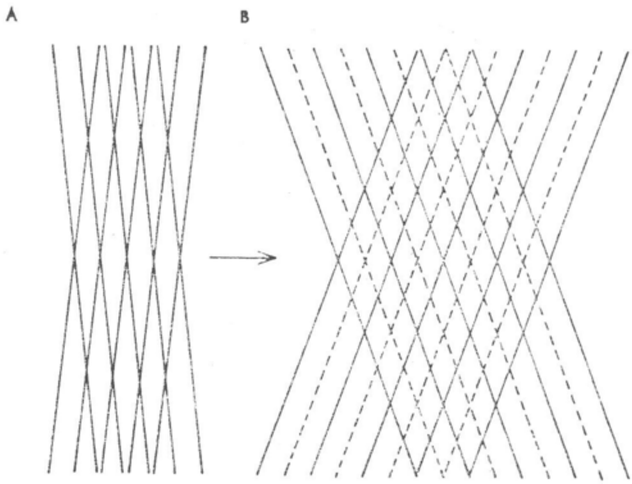

Fig. 6. Diagram illustrating the influence of radial growth on the arrangement of the crossed periclinal walls. A — initial stage, B — stage reached after radial growth. Dashed lines denote new walls, with the assumption that they are parallel to the walls of periclinally dividing cells

The wide-spread nonparallel alignment of periclinal walls implies that cambial initial cells from a three-dimensional network. A specific cambial initial can be in radial contact with various cells of the neighboring row at various levels, but only one of the cells in that row is an initial cell. Consequently initial cells of neighboring rows come into contact with one another at certain limited sites, elsewhere, however, they extend inward or outward from those contact sites. Along segments where the initials do not contact one another, radial growth causes their divergence. Geometrically the overall thickness of the network of initial cells depends upon the degree to which individual initials project from the mean plane of initial cells. This thickness would increase during the course of radial growth if this were not prevented by unequal periclinal divisions. The latter in effect cut off those parts of the initials that project from the mean layer. This cutting off of ends produces holes in the projection of initial cells on the cambial cylinder surface. These holes in turn are subsequently filled in by intrusive growth localized at the ends of the shorter initial cells produced by unequal periclinal divisions.

Because of their discontinuous contiguity, the periclinal walls of the initial cells of neighboring rows do not form a geometric layer. Consequently the concept of cambium as a single layer of initial cells may not have a physical basis since such a continuous, integral layer may not exist. A layer of initial cells need exist only in the ideal or functional sense — but need not be a geometric reality.

Toward the end of the period of seasonal cambial activity, the processes compensating for the lack of parallelism of neighboring initial cells must necessarily prevail over those promoting further crossing of cells. Because of this the gaps in the projection of the network of initials are gradually filled in and a continuous initial cell layer is restored. Cambium with nonparallel initial cells must, therefore, continually reconstruct the arrangement of its cells.

Acknowledgments

The author gratefully acknowledges the assistance of J. A. Romberger in the preparation of this paper.

This research was partially supported by a grant (FG-Po-297) from the U.S. Department of Agriculture.

REFERENCES

Barnett J. R., 1973. Seasonal variation in the ultrastructure of the cambium in New Zealand grown *Pinus radiata*. D. Don. Ann. Bot. 37: 1005-1011.

Luft J. H., 1961. J. Biophys. Biochem. Cytol. 9: 409.

Murmanis L., 1970. Locating the initial in the vascular cambium of *Pinus strobus* by electron microscopy. Wood Sci. Techn. 4: 1-17.

Murmanis L., 1971. Structural changes in the vascular cambium of *Pinus strobus* L. during an annual cycle. Ann. Bot. 35: 133-141.

Srivastava L. M., O'Brien T. P., 1966. On the ultrastructure of cambium and its vascular derivatives. I. Cambium of *Pinus strobus* L. Protoplasma 61: 257-276.

White P. R., Millington W. F., 1954. The structure and development of a woody tumor affecting *Picea glauca*. Amer. J. Bot. 41: 353-361.

Wilson B. F., Wodzicki T. J., Zahner R., 1966. Differentiation of cambial derivatives: proposed terminology. Forest Sci. 12: 438-440.
Włoch W., 1976. Cell events in cambium, connected with the formation and existence of a whirled cell arrangement. Acta Soc. Bot. Pol. 45: 313-326.

Nierównoległość komórek kambium w sąsiednich rzędach

Streszczenie

Ściany peryklinalne komórek kambium w sąsiednich rzędach mogą być nierównoległe, przy czym nierównoległość ta może być na tyle duża w okresie aktywności kambium, że pary komórek z sąsiednich rzędów kontaktują się tylko na pewnych odcinkach. Oznacza to, że komórki inicjalne zamiast ciągłej warstwy mogą tworzyć sieć z której, niektóre ich części wystają poza warstwę wzajemnych kontaktów. Nierównoległość komórek ulega powiększeniu w czasie symplastycznego wzrostu promieniowego. Przeciwdziałają temu nierówne podziały peryklinalne które w przypadku komórek inicjalnych obcinają części wystające poza warstwę wzajemnych kontaktów. W czasie przechodzenia kambium z okresu aktywności do okresu spoczynku odtwarza się ciągła warstwa komórek inicjalnych w czym odgrywa rolę wzrost intruzywny wydłużający komórki uprzednio skrócone w rezultacie nierównych podziałów peryklinalnych. Ściany podziałowe w komórkach kambium mogą być zawichrowane, to znaczy zmieniają swoją orientację w podłużnym przebiegu skręcając się jak śmigło samolotu. Ściana podziałowa będąca peryklinalną w pewnej części komórki może być antyklinalną w innej części.